# Biohydrogen

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Biohydrogen means the new strategies to reach green and sustainable hydrogen production and exploitation technologies.

green fuel biohydrogen microalgae

# 1. Introduction

Nowadays, searching for renewable energy represents one of the major challenges for the global scientific community. Population growth and industrial activities, with consequential high-energy demand, require solutions and proposals in the short-term. It is assumed that the world population could reach 8–10 billion in 2040, while the increase in global energy demand, within the same year, is estimated between 100 and 170 qBtu. However, models cannot accurately predict sudden changes in the global energy situation, as different socio-economic scenarios and political choices modulate each country's response <sup>[1][2]</sup>. Considering the slow oil, coal, natural gas formation processes, the excessive exploitation of fossil fuels has triggered a drastic and irreversible reserve reduction. Another side aspect of fossil fuel use concerns greenhouse gas (GHG) emissions, particularly carbon dioxide (CO<sub>2</sub>), during the combustion reaction <sup>[2]</sup>. In particular, in the years between 1998 and 2018, CO<sub>2</sub> emissions increased by 48%, making it necessary to implement carbon capture and storage (CCS) technology to limit serious and detrimental effects on climate change <sup>[2]</sup>. In this worrying scenario, fossil fuels still provide more than 80% of primary energy needs, although the interest in renewable sources, together with their consumption, are steadily increasing <sup>[4]</sup>.

Among renewable options, hydrogen shows the important advantage of  $CO_2$ -free combustion, with water as a byproduct. Thermodynamic properties, compared to traditional fuels, confirm the interest in its investments in research, although several aspects related to production and storage are still to be mastered. Today, several industrial applications depend on hydrogen, but the most is obtained by techniques, such as steam reforming or electrolysis, not entirely free from the involvement of fossil fuels <sup>[5]</sup>. Some living organisms, such as microalgae and bacteria, are the basis of processes capable of producing hydrogen in a completely eco-sustainable way. Microalgal hydrogen production is made possible by biological processes directly or indirectly, depending on sunlight, or by fermentation processes and thermochemical technologies for biomass conversion (Figure 1). Figure 1. Hydrogen production processes in microalgae.

In recent years, many green systems have focused on algal biomass to obtain energy from living matter. Microalgae store and exploit light energy thanks to the photosynthetic process. To support cell growth and metabolic activities, they use resources widely available in nature, such as water and  $CO_2$ . These organisms can concentrate considerable  $CO_2$  amounts and obtain nutrients necessary for growth, even from substrates or waters deriving from industrial waste. Algal cultivation systems, which are simple and relatively inexpensive, ensure advantageous growth rates. Furthermore, they can be set up on infertile territories without competing with agricultural areas that can be exploited for food resources. In particular, the general interest in microalgae has increased significantly in recent decades due to the variety and versatility of the metabolites present in various and numerous species <sup>[6]</sup>.

### 2. Fermentation Processes and Biomass-Applied Technologies

Direct and indirect biophotolysis processes are intrinsically linked to the photosynthetic process and the connected electron transport chain. Together with these two photosynthetic pathways that contribute to the production of hydrogen, another one linked to fermentative metabolism has also been identified. In dark conditions, the enzymatic activity of pyruvate:ferredoxin oxidoreductase (PFR) in Chlamydomonas reinhardtii is responsible for the reduction of Fd and the passage of electrons towards hydrogenase. Overall, this pyruvate-dependent hydrogen production acts in ways similar to those observed in bacteria, and though the yield is low, its contribution is not negligible  $\mathbb{Z}$ . The accumulation of complex carbohydrates, such as starch, or endogenous substrates, is positively associated with the production of hydrogen, while, it was also observed that exogenous carbon-rich media further stimulate its production in the early anaerobic stages. Several fermentative bacteria use anaerobic processes to transform carbon sources into various by-products, including hydrogen. Processes, such as photo- and darkfermentation, are commonly exploited and widely investigated in bacteria species, such as Escherichia coli, Clostridium spp., Thermococcales spp., Rhodobacter spp., and Rhodopseudomonas spp. [8]. The consumption of organic substrates, also deriving from waste, by photofermentation, include the transformation into organic acids, alcohols,  $CO_2$ , and  $H_2$  in presence of light, but with a low overall yield of solar energy conversion. In a similar way, but without light, dark fermentation uses various substrates and waste too, leading to the release of different components and gaseous mixtures in which hydrogen is present [10][11]. It has recently been observed that

hydrogen production can be increased by up to 60% compared to Chlamydomonas reinhardtii monoculture systems, by using co-culture systems with Escherichia coli. Growth media glucose-rich are exploited by bacteria that produce acetic acid, which can be used in algal metabolism <sup>[12]</sup>. Synergistically, different photobiological and fermentative microbial metabolisms may interact and cooperate increasing the hydrogen yields <sup>[13]</sup>.

Microalgae show an enormous biodiversity being present in different habitats, even extreme and hostile to most living organisms, suitably adapting their metabolism. It is therefore possible to modulate the growth conditions to obtain biomass of the desired composition, based on the requirements of the downstream process also using industrial and agricultural processing water and waste. In this perspective, various strategies have been applied by combining bioenergy production and bioremediation approaches <sup>[14]</sup>[15]. Unicellular green alga Scenedesmus obliquus managed to biodegrade the phenolic content present in the olive oil mill wastewater. This strategy makes it possible to remedy a problem particularly encountered in the Mediterranean area and, since the biotransformation carried out consumes oxygen, favorable conditions to trigger a concomitant production of hydrogen are also generated <sup>[16]</sup>. A consortium of microalgae, mainly composed of Scenedesmus and Chlorella species, grown in pig manure showed good growth, without the addition of external nutrients, and significant fermentative hydrogen production <sup>[17][18]</sup>.

One of the main problems associated to these approaches is the elevated costs in terms of management and purification of the components obtained, which include the presence of several by-products, also toxic. Moreover, although the biomass of the microalgae contains a reduced lignin content compared to other lignocellulosic feedstock previously used for energy purposes, preliminary treatments are often necessary to facilitate the extraction and conversion of the microalgae content. Mechanical, thermal, chemical, or biological treatments are often applied to biomass separately or in combination as a preliminary step <sup>[1][2]</sup>.

## 3. Conclusions

Hydrogen is undoubtedly the future fuel for its green and environmentally friendly properties. However, its production technology is still based on fossil fuel; thus, carbon releasing. Scientific research is working hard to improve new strategies to reach green and sustainable hydrogen production and exploitation technologies.

Microalgae seem to be an attractive solution to this problem. As previously described, they can produce biological hydrogen without carbon emissions; rather, by fixing it during the process. The limiting factor for large-scale applications of this ability is that of low production yield, and, therefore, scientific research must focus in this direction. Solutions described in this review represent the most promising developments for implementing hydrogen yield.

The extreme versatility of microalgae also consents to combine several applications; thus, multiplying the benefits. The use of microalgae in dedicated fuel cells allows the development of an ecological energy production system, which can be associated with bioremediation advantages. In fact, microalgae can grow even in wastewater, purifying them from heavy metals and other dissolved substances. Since current wastewater treatment plants present some critical issues concerning GHG emissions <sup>[19][20][21]</sup>, developing an integrated purification and energy production facility based on microalgae could represent a promising ecological technology for the future.

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